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Effects of the Algal Toxin Microcystin on Fishes in the James River, Virginia

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**EFFECTS OF THE ALGAL TOXIN MICROCYSTIN ON FISHES IN
THE JAMES RIVER, VIRGINIA**

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science in Biology at Virginia Commonwealth University.

by

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List of Abbreviations

AAE: *Alosa aestivalis*

ASA: *Alosa sapidissima*

DCE: *Dorosoma cepedianum*

DPE: *Dorosoma petenense*

DW: Dry Weight

H: High Concentration Site (Rice Rivers Center)

HAB: Harmful Algal Bloom

HLNFH: Harrison Lake National Fish Hatchery

L: Low Concentration Site (Curles Neck Creek)

MC: Microcystin

Abstract

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By Maxwell Douglas Haase, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2015

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Director, Center for Environmental Studies; Associate Professor, Department of Biology;
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With the global rise in frequency of harmful algal blooms in estuarine environments comes an increase in prevalence of toxic metabolites, such as microcystin (MC), that some of the cyanobacteria involved will produce. At high concentrations, MC may accumulate in consumer tissues and have deleterious effects on organisms; however impacts of the toxin on aquatic living resources at ecologically relevant concentrations have not been widely documented. We analyzed the effects of MC on juveniles of five fish species from the James River, Virginia to determine if

MC has the potential to impede growth. Using three separate experimental approaches, it was shown that exposure to concentrations of the toxin currently observed in the James River estuary do not appear to significantly impact the growth or survivorship of tested fish species. Extraneous factors in parts of the study led to an inability to draw clear conclusions on mortality or growth impacts; however it is evident from the experiments that at least some of the fish species have biological mechanisms in place that allow them to effectively eliminate the toxin from their systems. An ability to extricate the toxin suggests the possibility for fishes to withstand MC exposures and sustain few negative health impacts at low MC concentrations.

Introduction

Harmful algal blooms (HABs), and the related cultural eutrophication of surface waters, are a growing concern globally (Anderson et al., 2008; O'Neil et al., 2012). HAB events have been shown to cause restrictions on human drinking water supplies (Wilson, 2014), inundate shorelines with algae (Song et al., 2014), and kill aquatic organisms in the vicinity of blooms (Imai and Yamaguchi, 2014). Research suggests that eutrophication and rising temperatures of surface waters may promote the expansion and proliferation of harmful algae, resulting in a likely increase in magnitude and frequency of HABs in the future (O'Neil et al., 2012).

Future increases in HABs are particularly concerning in tidal estuaries where significant cultural and economic losses could result from major bloom events. Estuaries provide ideal conditions for algal proliferation which may increase the likelihood of HAB events in coastal bodies of water. The Chesapeake Bay, the largest estuary in the U.S.A., is a coastal body of water that has been substantially altered by increased cultural eutrophication (Boesch et al., 2001). The Chesapeake Bay is fed by many large, tidal rivers which have physical characteristics that make them susceptible to HAB events. One such tributary, the James River, Virginia, has been observed to have large nutrient inputs which

may have been conducive to the HABs previously documented in the system. (Wood et al., 2014).

Some algal taxa associated with HAB events may produce toxic metabolites that can have harmful effects on living aquatic resources (Carmichael, 1992). One group of metabolites, microcystins (MCs), are hepatotoxins that may cause acute poisonings as well as cancer promotion following chronic exposures (de Figuieredo et al., 2004; Ernst et al., 2006; Pavagadhi and Balasubramanian, 2013). Cyanotoxins have been documented in the tissues of many aquatic taxa including mussels, crustaceans, and fish (Amorim and Vasconcelos, 1999; Vasconcelos et al., 2001; Zimba et al., 2001; Zimba et al., 2006; Malbrouck and Kestemont, 2006; Gobble and Kudela, 2014; Wood et al., 2014). Mortality from MC toxicosis has also been shown in tertiary consumers as evidenced by 21 MC-linked mortalities involving southern sea otters in Monterey Bay, California (Miller et al., 2010).

Controlled laboratory studies have successfully demonstrated the negative health effects of MC exposure on various fish species. In a synthesis of MC effects, acute exposure to the toxin was typically associated with high incidence of liver necrosis, impaired reproductive capabilities, reduced growth rates, and mortality in fish (Malbrouck and Kestemont, 2006). A large proportion of the cited experiments were performed using MC concentrations higher than documented natural occurrences and using artificial uptake pathways (e.g. intraperitoneal injection and gavaging). In contrast, few published studies demonstrate the effects of MCs when fish are subjected to ecologically pertinent

concentrations of the toxin through natural uptake pathways (i.e. dietarily). Results from the ecologically relevant studies range from no discernable impacts to severe symptoms associated with MC toxicosis (Malbrouck and Kestemont, 2006; Acuna et al., 2012).

The objective of our study was to evaluate the potential impact of ecologically relevant concentrations of the cyanotoxin MC on vulnerable components of estuarine fish assemblages in the James River, Virginia. It was hypothesized that subjecting fish to MC concentrations similar to concentrations observed during James River HAB events would negatively impact the welfare and survivorship of the fish. We conducted three experiments in order to accomplish our objective. Multiple test species were used in the experiments as well as different modes of exposure. We used *in situ* enclosure experiments to assess growth and mortality associated with estuarine HAB events on juvenile Clupeids, laboratory exposure to assess viability and mortality rates of *Alosa* spp. eggs and larvae, and examined effects on the growth and mortality rates of Atlantic Sturgeon dietarily exposed to incrementally increasing concentrations of the toxin.

The embryonic, larval, and juvenile development of various fish species were examined due to a higher susceptibility to toxicosis at these critical life stages (Malbrouck and Kestemont, 2006; Mohammed, 2013). The species chosen were primarily from the family Clupeidae, which are pelagic, planktivorous fish during juvenile life stages. The trophic designation of the juvenile Clupeids makes them highly susceptible to encountering and consuming MC producing algae during HAB events (Stone and Daborn, 1987; Eagles-Smith et al., 2008). The clupeid species studied include Blueback Herring (*Alosa*

aestivalis), American Shad (*A. sapidissima*), Gizzard Shad (*Dorosoma cepedianum*), and Threadfin Shad (*D. petenense*). All four species spawn in the spring and undergo development during the summer months when estuaries are most prone to HABs (Anderson et al., 2008). A single benthic species, Atlantic Sturgeon (*Acipenser oxyrinchus*), was chosen due to its endangered status (50 CFR 224.101) making it a species of concern. While Atlantic Sturgeon are less likely to actively feed on MC producing algae, the fish still have the potential to encounter MC as secondary consumers feeding on benthic invertebrates that have accumulated the toxin (McLean et al., 2013; Miller et al., 2010; Wood et al., 2014).

Methods

Study Locations

Experiments involving *Alosa* spp. (Blueback Herring and American Shad) eggs and larvae were conducted at Harrison Lake National Fish Hatchery (HLNFH). Blueback Herring eggs were sourced from manual field collections in the adjacent stream, Herring Creek, and American Shad eggs were provided by HLNFH.

Experiments involving *in situ* enclosures were conducted in the tidal James River, Virginia using juveniles from three different Clupeid species: Gizzard Shad, Threadfin Shad, and Blueback Herring. The estuary is located in the U.S. mid-Atlantic region and extends 115 km from the Fall Line at Richmond to the Chesapeake Bay at Hampton Roads. The estuarine portion of the James River is conducive to HAB events as it is a warm, shallow (~3m), and eutrophic estuary that receives large anthropogenic nutrient loads (Wood et al., 2014). The *in situ* enclosures were located at two sites on the James River: the Rice Rivers Center and Curles Neck Creek (Figure 1). The Rice Rivers Center site was chosen because the area has been shown to have high MC concentrations in the late summer months (Wood et al., 2014). The Curles Neck Creek site was chosen because it was assumed to have lower MC concentrations than the Rice

Rivers Center site. It is upstream and more channelized than the Rice site, resulting in lower phytoplankton abundance (Bukaveckas et al., 2011).

The dietary exposure experiments using Atlantic Sturgeon juveniles were conducted in the aquatics facility at VCU's Trani Center for Life Sciences. The lab has multiple large holding tanks capable of housing the Atlantic Sturgeon, as well as experimental tanks in which the studies were conducted.

Alosa spp. Egg and Larvae MC Exposure Experiments

Eggs and larvae were subjected to target MC concentrations of 0.0, 0.4, 0.8, and 1.6 $\mu\text{g MC}\cdot\text{L}^{-1}$. The MC concentrations used were based on observed maximum concentrations in 2012 from the James River (Wood et al. 2014). A total of eight egg and eight larvae replicates for each dosage treatment were performed. Six egg and six larvae replicates were performed using Blueback Herring eggs and larvae and two replicates used American Shad eggs and larvae.

Blueback Herring eggs were acquired by manually strip spawning the fish during their spring migration. Fertilized eggs were taken to Harrison Lake National Fish Hatchery (HLNFH) and placed in rearing tanks. Eggs were rested for 24h post-fertilization, then visually inspected for viability. Egg MC exposures were accomplished within closed circulation-filtration systems. The systems used followed a design originally developed for freshwater mussel propagation (Barnhart, 2006).

The design used for the egg trials consisted of a 3 gallon bucket (11 L) inlaid in a 5 gallon bucket (18 L) with six holes cut out of the bottom of the 3 gallon bucket. The holes were arranged with a center hole housing a pump to keep water flowing throughout the system and five holes around the perimeter of the bucket housing five egg containment capsules. The egg containment capsules were comprised of PVC and 200 μm Nitex screen. The containment capsules provided chambers (51 mm diameter) in which eggs could be held and later recovered while allowing water to flow past the eggs (Figure 2).

The water used in the buckets was supplied from an underground well at HLNFH that has a constant temperature of 17°C. ELISA analysis of the well water did not detect the presence of MC. Temperature was maintained in the systems using a flow-through water bath that had a constant supply of well water (Figure 2). Water MC concentrations were tested for each treatment from the first egg trial and observed concentrations were within 20% of target concentrations. The values were deemed to be adequately within range of target concentrations, therefore water testing for other trials was not considered necessary. For each experimental trial, ten eggs were placed into each egg containment capsule such that 100 eggs were used for each treatment, including the control. Each treatment was dosed with the appropriate concentration (0.0, 0.4, 0.8, or 1.6 $\mu\text{g MC}\cdot\text{L}^{-1}$) and after 48h eggs were sorted for viability (no whitening of egg and embryo intact) and counted.

Larval trials were conducted using 5 gallon buckets for the treatments. Well water was used in the buckets as well as the same flow-through water bath that was used in the egg trials. Each of two replicate buckets was stocked with 50 yolk-sac larvae for a total of 100 larvae per

treatment. After the 48h exposure, the live proportion of fish was counted and percent survivorship was calculated. Due to the difficult nature of counting the larvae without inducing further mortality, multiple techniques were attempted to count the remaining viable larvae during the first larvae trial. The most effective method found was a “dead-count” method in which only dead fish were counted and survivorship was calculated by subtracting the counted value from the original total (50).

Alterations to the methods occurred in the second and third Blueback Herring larvae trials to determine if the high mortalities in the first Blueback Herring trial were due to handling stress from the counting methods used. The changes included adding an additional replicate to the second trial for a total of 150 specimens per treatment, and including a 5th treatment concentration (3.2 µg MC L⁻¹) in the third trial (Table 1).

James River *In Situ* Enclosure Experiments

Cylindrical enclosures (~0.8 m³), constructed from 1 cm² Nytex mesh and a PVC pipe frame, were deployed at two sites in the James River (Figure 1) to assess fish response to natural concentrations of MC for known periods of time. Species representing the family Clupeidae were used for enclosure deployments. The first deployment used juvenile Gizzard Shad from HLNFH. The source for Gizzard Shad juveniles was depleted after the initial experimental trial. A second deployment used juvenile Threadfin Shad electrofished from the James River near Richmond, Virginia. The third deployment used stocked Blueback Herring from HLNFH. A subsample of

15 fish was analyzed for liver MC concentrations prior to each deployment. Fish returned levels below the detectable limit of the assay.

Two enclosures were positioned at each of the two sites (Figure 1) and were stocked with 30 fish each, yielding a total 60 fish per site. Deployments lasted 14 days and the experiment was replicated 3 times during the period of Aug. 9 - Sep. 20, 2013 to correspond with predicted HAB events. At the end of each deployment period, all fish were removed from the enclosures and euthanized in adherence to established VCU-IACUC protocols. The collected post-exposure specimens were taken back to the lab where length and mass measurements were obtained and the livers from 15 randomly selected fish from each replicate were removed for MC analysis. For the duration of the experiments, enclosures were checked daily to remove any deceased specimens. Ambient water MC concentrations were monitored at the two sites for the duration of the experiments.

Dietary Exposure of Atlantic Sturgeon to MC

Diet Formulation

The feeding methods used for dietary exposures were modified from Hirschfeld et al. (1970) to allow incorporation of chemical toxins with a semi-solid food matrix. Pellet formulation minimized fragmentation of pellets and MC leaching in closed-system tanks during fish feeding bouts. MC powder (0.5 mg MC-LR from Abraxis BioScience) was added to 125 mL of deionized water to yield a 4 µg/mL solution of MC stored at 4°C. The diets were prepared by

thoroughly mixing 250 g of fish feed (Melick Aquafeed sinking No. 1 pellets, Melick Aquafeed, Catawissa PA, USA), 20 g granulated agar, 14 g unflavored gelatin, and MC solution (0 mL, 8 mL, 16 mL or 32 mL, depending on treatment group) to 1000 mL of deionized water heated to 100°C. The mixture was allowed to cool for 24 h in a flat pan at 4°C and then cut into pellets of a size (1-3 mm²) appropriate for ingestion by age-0 Atlantic Sturgeon. Prepared pellets were placed in labeled containers and stored at -20°C for less than 1 week prior to experimentation. Four separate diets corresponded to the following treatment groups: 0.000 (control), 0.014, 0.028, 0.057 ± 0.011 µg MC·g⁻¹ DW of food. The range in MC concentrations in the formulated diet encompass MC tissue concentrations in James River benthic invertebrates, including *Rangia* clams (\bar{x} = 0.025 µg MC·g⁻¹ DW)(Wood et al. 2014), which are potential prey for wild juvenile Atlantic Sturgeon in the James River (Scott and Crossman, 1973). Concentrations of MC in dietary treatments were measured prior to experimentation.

Leaching Experiment

A pellet leaching experiment was conducted to test the assumption of no loss of MC from pellets during feeding bouts. Five g of pellets (0.057 µg MC·g⁻¹ DW) were placed in 1.0 L of deionized water and aliquots (20 ml) of water removed at regular intervals for a period of 12 h after the food was first submerged. The solution was stirred gently before each sample was taken. Analysis of the samples showed that the pellets retained 94% of initial MC concentrations after 15 minutes, which was the time designated for Atlantic Sturgeon feeding bouts (the time necessary for fish to consume all pellets). Only 47% of MC in the pellets was retained after 12 h,

but the 12 h leaching effect was not relevant to the Sturgeon experiment. The drastic leaching over time should be noted if attempting to replicate our diet formula and it is suggested that food not be left in solution for extended periods or leaching effects may occur.

Experimental Animals

Approximately 200 age-0 (40-100 mm FL) Atlantic Sturgeon from a Canadian wild source (St. Lawrence River) were secured under an agreement between VCU and the Maryland Department of Natural Resources, transferred to VCU's Aquatics Facility (1000 W. Cary Street, Richmond VA), and acclimated in freshwater (< 0.5 ppt) at 16°C to the formulated artificial diet containing no MC for 1 month prior to the start of the experiment. Fish were held and used under conditions established by VCU's IACUC committee (protocol AD10000441).

MC Exposure

A single experimental system consisted of a clean, 38-liter glass tank (closed system) with a single age-0 Atlantic Sturgeon. Water was sourced from the VCU Aquatics Lab supply (UV and chlorine filtered city water). Treatment groups were fed pellets containing 0.00 (control), 0.014, 0.028, or $0.057 \pm 0.011 \mu\text{g MC} \cdot \text{g}^{-1} \text{DW}$ of food daily for the duration of the experiment (28d). All other conditions (e.g. water quality, temperature, photoperiod) were consistent among treatments. Each treatment group consisted of 7 replicates, for a total of $n=28$ experimental units. Mass (to nearest 0.01 g; blotted wet weight) and fork length (mm) of each

fish were measured and recorded during transfer from the stock tank to experimental tanks prior to the initial experiment feeding. Water changes of 50% were completed every 2 days, or as needed, for the duration of the experiment and solid wastes were siphoned from the tanks daily. All fish (experimental and control) were fed a daily ration of approximately 3% of the mean body mass of the fish initially selected for testing. Feeding occurred once per day with the appropriate treatment diet at about 1 PM. The daily ration was presumed to be a maintenance (zero net growth) ration for juvenile sturgeon (Albert Spells, USFWS, Charles City VA, personal communication) held at room temperature.

Exposure period (d) and daily consumption of pellets were recorded for each fish; the data was used to calculate total (cumulative) MC consumption (μg) for each fish in a non-control treatment during its deployment period. The feeding experiment proceeded for 28 consecutive days, after which all remaining fish were euthanized, evaluated, and stored at -20°C . Livers were removed from each fish within 1 week of the conclusion of the experiment and analyzed for MC concentration.

Microcystin Analysis

All MC concentrations in water, food, and fish tissue samples were analyzed using an ADDA ELISA kit (detection limit $0.05 \mu\text{g L}^{-1}$; Abraxis; Warminster PA, USA) using the methods of Wood et al. (2014). The assay measures numerous forms of free MCs using polyclonal antibodies. Measured concentrations are reported in MC-LR equivalents. All samples

were run in duplicate and MC concentration ($\mu\text{g L}^{-1}$) was expressed as the arithmetic mean of the two values. MC values below the analytical detection limit were assigned a value of zero.

Statistical Analysis

All data was entered into an EXCEL spreadsheet and with a double-entry method prior to analysis. Non-parametric statistical analysis was performed for all data. Statistical methods were developed using Ott and Longnecker (2001) and processed using the R statistics program (2014). Kruskal-Wallis tests are reported showing the chi-squared test statistic and the associated p-value using the degrees of freedom (shown in parentheses) for the particular analysis. Wilcoxon tests report only p-values for the two samples being compared. The significance level for all statistical analyses was $\alpha = 0.05$.

For the *Alosa* spp. egg and larvae trials, analysis of variance was performed using Kruskal-Wallis tests. The tests were run for each of the eight egg and larvae trials to determine if the varying treatment doses in each trial caused any differences in viability or survivorship for that trial. No post-hoc analysis was required for the eight Kruskal-Wallis tests that were performed.

For the *in situ* enclosure experiments, analysis of variance using a Kruskal-Wallis test was performed to compare the three separate trials to one another, and determine if differences in MC uptake occurred between trials. Post-hoc tests were required for the analysis and were performed using the Wilcoxon rank sums test with Bonferroni correction. Wilcoxon tests were

also used for two-sample comparisons within individual trials, allowing comparisons between MC uptake at the Rice Rivers Center site and MC uptake at the Curles Neck Creek site. The two-sample Wilcoxon test was performed for each of the 3 trials.

For the Atlantic Sturgeon dietary exposures, analysis of variance was performed for three different comparisons using a Kruskal-Wallis test for each. The first compared toxin exposure time between treatment groups, the second compared liver MC concentrations between treatment groups, and the third compared changes in somatic mass between treatment groups. Post-hoc analysis was not required for the three Kruskal-Wallis tests performed for the experiment. Three linear regressions were also performed for the Atlantic Sturgeon exposures to compare total MC consumption with liver MC concentrations, total MC consumption with changes in somatic mass, and total food consumption with changes in somatic mass.

Results

Alosa spp. Egg and Larvae MC Exposure Experiments

We found no statistically significant impact on the viability of *Alosa* spp. eggs or larvae when subjected to MC concentrations of 0.4 – 0.8 µg/L. Both eggs and larvae retained a 90% or higher viable proportion across nearly all trials and MC dosage concentrations (Table 1). The only outliers were the 0.8 and 1.6 µg/L larvae groups for the first Blueback Herring trial. The highest dosage concentration was doubled for the final Blueback Herring larval trial and the resulting viable proportion was 1.0 for the new concentration (3.2 µg/L), indicating that the low viable proportions found in the first Blueback Herring trial were likely due to handling stress. Kruskal-Wallis tests performed for each of the eight individual egg and larvae trials showed no significant difference between viable proportion and dosage concentration ($\chi^2(3,4) < 6.0$ and $p > 0.1$ for each).

James River *In Situ* Enclosure Experiments

Mortality within enclosures was problematic for some of the *in situ* replicates. Only the Gizzard Shad deployment retained the target number of fish per replicate ($n = 15$) after 14 days. Retrieval for the Threadfin Shad deployment ranged from 4 to 14 fish per replicate and retrieval for the Blueback Herring deployment ranged from 0 to 4 fish per replicate (Table 2). A Kruskal-

Wallis test revealed a statistically significant difference in liver MC concentrations when comparing the three separate trials [$\chi^2(2) = 19.9$, $p < 0.001$]. Post-hoc Wilcoxon analysis indicated the liver concentrations from the Gizzard Shad trial were significantly higher than the other two trials (Threadfin Shad – $p < 0.001$; Blueback Herring – $p = 0.016$). Post-hoc indicated no difference between Threadfin Shad and Blueback Herring liver concentrations ($p = 0.33$).

Measured ambient MC concentrations in the James River for the duration of the deployments (Aug 9 – Sep. 20, 2013) were significantly higher at the high concentration site (H) (Rice Rivers Center; $n = 7$) than at the low concentration site (L) (Curles Neck Creek; $n = 3$) ($\bar{x}_H = 0.297 \pm 0.043 \mu\text{g MC}\cdot\text{L}^{-1}$, $\bar{x}_L = 0.060 \pm 0.013 \mu\text{g MC}\cdot\text{L}^{-1}$, $p = 0.017$). The difference between ambient water MC concentrations from the two sites was only reflected in liver MC concentrations for the Threadfin Shad deployment ($\bar{x}_{H_{\text{Threadfin}}} = 0.156 \pm 0.068 \mu\text{g MC}\cdot\text{g}^{-1} \text{ DW}$; $\bar{x}_{L_{\text{Threadfin}}} = 0.013 \pm 0.010 \mu\text{g MC}\cdot\text{g}^{-1} \text{ DW}$, $p < 0.01$). The Gizzard Shad deployment yielded significantly higher liver concentrations from the enclosures deployed at the low concentration site. ($\bar{x}_{H_{\text{Gizzard}}} = 0.125 \pm 0.048 \mu\text{g MC}\cdot\text{g}^{-1} \text{ DW}$; $\bar{x}_{L_{\text{Gizzard}}} = 0.477 \pm 0.093 \mu\text{g MC}\cdot\text{g}^{-1} \text{ DW}$; $p < 0.001$). All liver MC concentrations for the Blueback Herring trial were below reportable concentrations ($< 0.001 \mu\text{g MC}\cdot\text{g}^{-1} \text{ DW}$).

Dietary Exposure of Atlantic Sturgeon to MC

High mortality rates among test subjects resulted in lower than target MC exposure times for all treatments (28d/specimen). A Kruskal-Wallis analysis revealed no significant difference between toxin exposure time and treatment group (control included) [$\chi^2(3) = 4.037$, $p = 0.258$]

suggesting that the mortality rates were not a function of time exposed to the toxin, but instead due to extraneous factors. Each treatment group was fed a different MC concentration in its diet, which resulted in an incrementally increasing mean total consumption of the toxin as treatment groups went from low to high dosage concentrations (Table 3).

Despite the increase in total MC consumed at higher dosage concentrations, Kruskal-Wallis analysis showed no significant differences in liver MC concentrations between treatment groups [$\chi^2(2) = 2.765$, $p = 0.251$]. A linear regression comparing total MC consumed and liver MC concentrations ($R^2 = 0.071$, $p = 0.065$) indicated that the amount of MC consumed is not a good predictor of liver MC concentrations in the tested year-0 Atlantic Sturgeon (Figure 3). It should also be noted that, despite a single outlier, the highest dose concentration yielded liver MC concentrations comparable to the liver concentrations found in the low and medium dose treatment groups despite the fish consuming more MC overall.

There was no significant difference between treatment groups and changes in somatic mass per day [$\chi^2(2) = 2.765$, $p = 0.251$]. All fish experienced a net loss in mass despite observed consumption of the administered feed. Possible causes for net loss in somatic mass in all fish may include residual water on the fish during initial measurements and desiccation due to freezer storage after death. Increased MC consumption was positively associated with a reduced loss in mass per day ($R^2 = 0.456$, $p < 0.001$) (Figure 4). A stronger association ($R^2 = 0.703$, $p < 0.001$) was found between total food consumed and reduced loss in somatic mass per day (Figure 5), suggesting that overall food consumption had a larger impact on changes of somatic mass in the test subjects than did MC concentrations in the diet.

Discussion

We were unable to detect any discernable deleterious impacts on the mortality and growth of juvenile test species when subjected to MC concentrations that are ecologically pertinent to the James River system during non-bloom years. The first set of experiments revealed no significant impact to *Alosa* spp. when exposed to MC concentrations more than three times the mean recorded levels from the James River in Summer 2012 (Wood et al., 2014). Data from 2011 through 2013 reveal only a single set of MC measurements from the James River on Sept. 6, 2011 (max = $4.67 \mu\text{g MC}\cdot\text{L}^{-1}$) that were above test concentrations (P. Bukaveckas, VCU, unpubl. data). The date in which these values were taken does not correspond with the spring spawning seasons for Blueback Herring or American Shad so it can be assumed that the eggs and larvae for the two species are not likely to encounter MC concentrations of a magnitude greater than the concentrations tested in the James River estuary. It has been shown that MC concentrations up to $50 \mu\text{g MC}\cdot\text{L}^{-1}$ have no discernable impact on the embryonic development in zebra fish (*Danio rerio*), however exposures as low as $5 \mu\text{g MC}\cdot\text{L}^{-1}$ resulted in 40% lower survivorship for larvae pre-exposed to the toxin during embryonic development (Oberemm et al., 1997). The potentially detrimental MC concentrations observed in the study are only slightly higher than recorded values in the James River indicating the toxin has the potential to cause negative impacts on fish larvae survivorship during large HAB events.

An important finding for the enclosure experiments was that the juvenile Gizzard Shad previously unexposed to MC exhibited rapid uptake of the toxin and multiple fish had liver concentrations higher than samples of wild caught juvenile Gizzard Shad (P. Bukaveckas, VCU, 2013 unpubl. data). Fish normally eliminate MC rapidly from their bodies (Dyble et al., 2011) so for previously unexposed fish to demonstrate higher than average retention rates suggests that extrication of the toxin begins after a certain threshold for MC exposure has occurred. Liver MC accumulation was not accompanied with elimination in Nile tilapia, *Oreochromis niloticus*, at dietary MC concentrations lower than $0.15 \mu\text{g MC}\cdot\text{fish}^{-1}\cdot\text{day}^{-1}$ (Deblois et al., 2011). The Gizzard Shad in our experiment may not have been subjected to high enough concentrations of the toxin through daily dietary intake or through ambient water concentrations to begin effective elimination of the toxin. Rapid MC uptake by tested fish could also be a result of being confined to feeding in the water column by the enclosures, whereas wild Gizzard Shad would be feeding on benthic sediments with low MC concentrations.

The enclosure experiments were unable to confirm that MC exposure was a contributing factor in mortality rates for the Blueback Herring and Threadfin Shad deployments. The Blueback Herring may have been too small for the enclosures, as a few of them were found having gilled themselves in the enclosure mesh. The cause of the high mortality in Threadfin Shad was not evident. Due to the high variability in MC uptake across all replicates, it was not possible to determine whether high MC uptake rates were a direct function of ambient MC concentrations or if extraneous factors contributed to the high uptake in certain enclosures.

The sturgeon experiment was also unable to confirm MC as the cause of mortality in the tested fish. Uncertainty regarding the cause of mortality was primarily due to a fungal infection in the sturgeon population increasing stress on the test specimens and preventing an optimal test procedure. The toxin may have been an additional stressor contributing to the high mortality in the fish however no inferences can be made. MC in the pellet diet did not appear to have any impact on fish feeding behavior and growth rates were highly associated with whether or not the individual fish were well enough to eat during feeding bouts. A significant finding from the dietary exposure experiment was that liver MC concentrations in the sturgeon did not follow the predicted dose response wherein higher quantities of the toxin consumed yield higher MC concentrations in the liver. The fish fed the highest dosage ($0.057 \mu\text{g MC}\cdot\text{g}^{-1}$) had liver MC concentrations that weren't statistically different from liver MC concentrations in fish subjected to the low or medium dosages (0.014 and $0.028 \mu\text{g MC}\cdot\text{g}^{-1}$). Despite the high dosage fish consuming the most MC overall, the fish had lower mean liver MC concentrations than the medium dosage fish (Table 3). The observation once again suggests that extrication of the toxin may have been triggered once exposed to a greater concentration of MC. The MC concentrations at which the Atlantic Sturgeon appeared to begin elimination of the toxin are lower than concentrations observed for Nile Tilapia (Deblois et al., 2011), suggesting that either Atlantic Sturgeon may have a greater capacity for elimination of MC than Nile Tilapia or other factors caused the high dosage subjects to not uptake the toxin as desired.

Based on the research that was conducted, concentrations of MC found in the James River may not pose a threat to the growth and development of tested juvenile fishes in the river.

Fish have evolved alongside MC producing cyanobacteria and previous studies suggest that fish have the ability to efficiently eliminate the toxin from their systems. However, research has repeatedly shown that high concentrations of the hepatotoxin can be damaging to fish physiologically (Malbrouck and Kestemont, 2006). Even though current MC concentrations encountered in the James River do not appear to be detrimental to fish survivorship, potentially harmful concentrations of the toxin could be encountered in the future. Monitoring of the estuary should continue in an effort to ensure the welfare of economically and ecologically important fishes in the James River, Virginia.

Figures and Tables

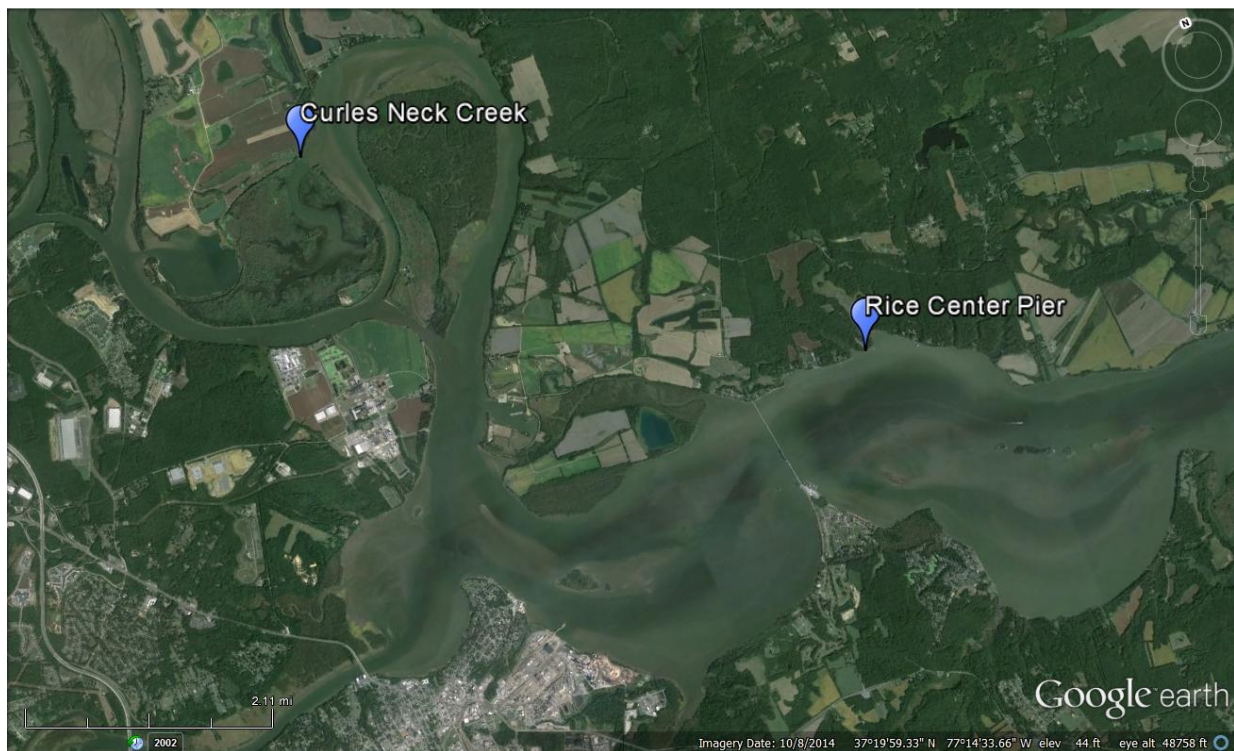


Figure 1. Map Indicating Enclosure Study Sites. Map of the two sites used for the *in situ* enclosure studies on the James River, Virginia. The sites are located on the river adjacent to the city of Hopewell, Virginia



Figure 2: System Used for Egg MC Exposures. Pictures of the system used in the *Alosa* spp. Egg experiments. The individual experiment system (left) shows the layout of the pump and egg containment capsules. All treatment replicates for a single egg trial are shown (right) with all experimental systems in a flow-through water bath. The water bath was also used for *Alosa* spp. larval trials

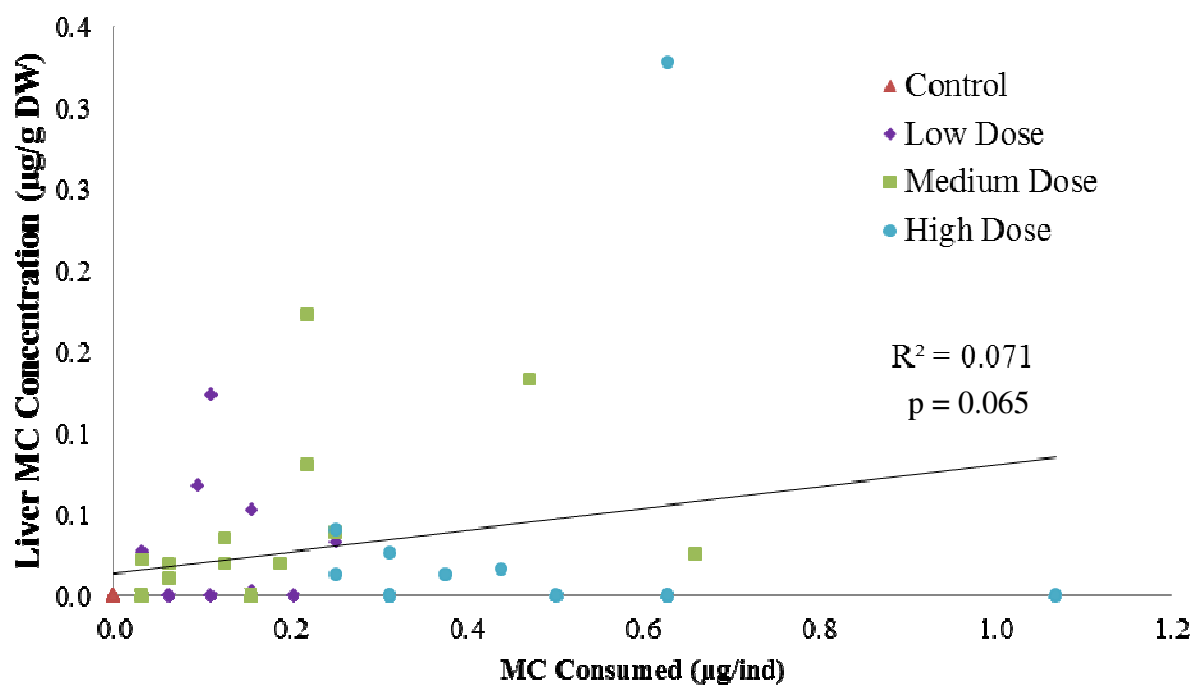


Figure 3. Atlantic Sturgeon Liver MC Concentrations and MC Consumption. Relationship between total consumption of MC (µg) and the concentration of MC (µg/g DW) in liver tissue samples for n=48 captive, juvenile Atlantic Sturgeon.

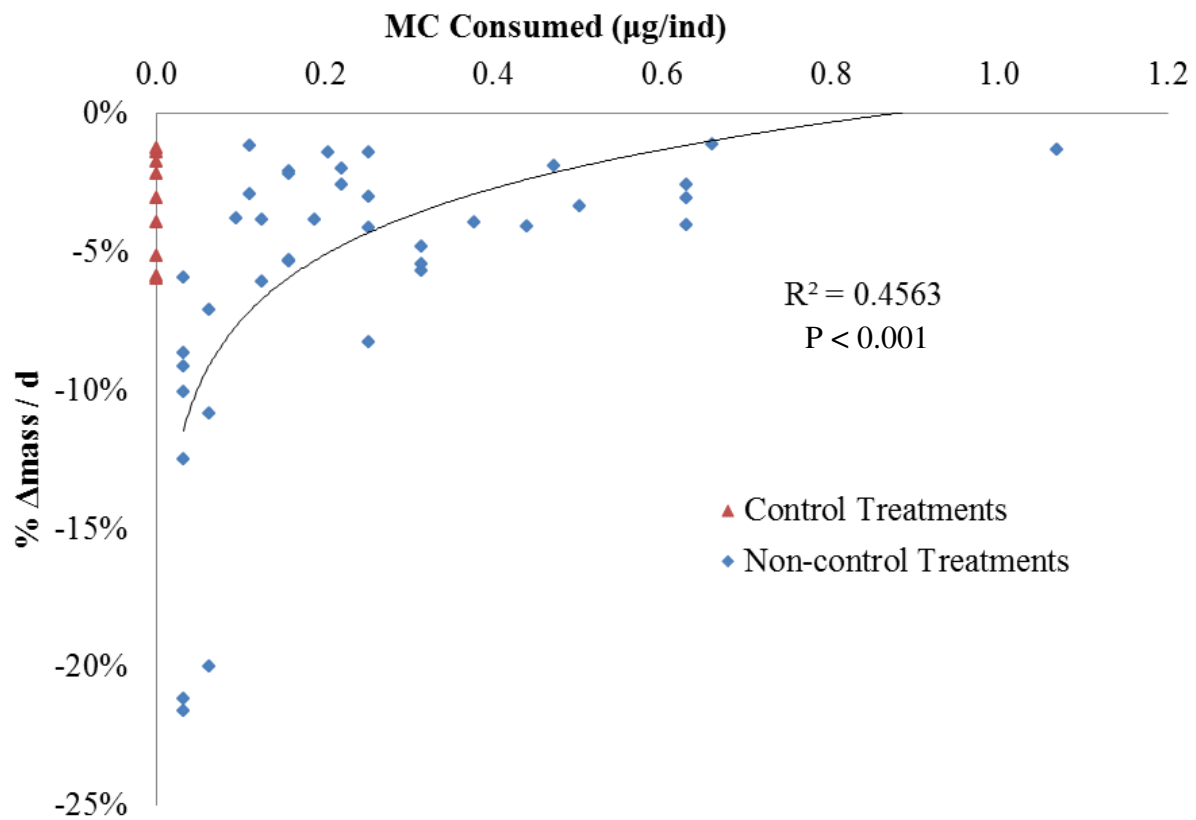


Figure 4. Atlantic Sturgeon MC Consumption and Change in Mass. Relationship between the calculated loss of somatic mass per day and the consumption of MC (µg) for n=39 captive, juvenile Atlantic Sturgeon in non-control treatments. Control treatment visualized for comparison (n=9) but not represented in the R^2 due to zero values for estimated intake.

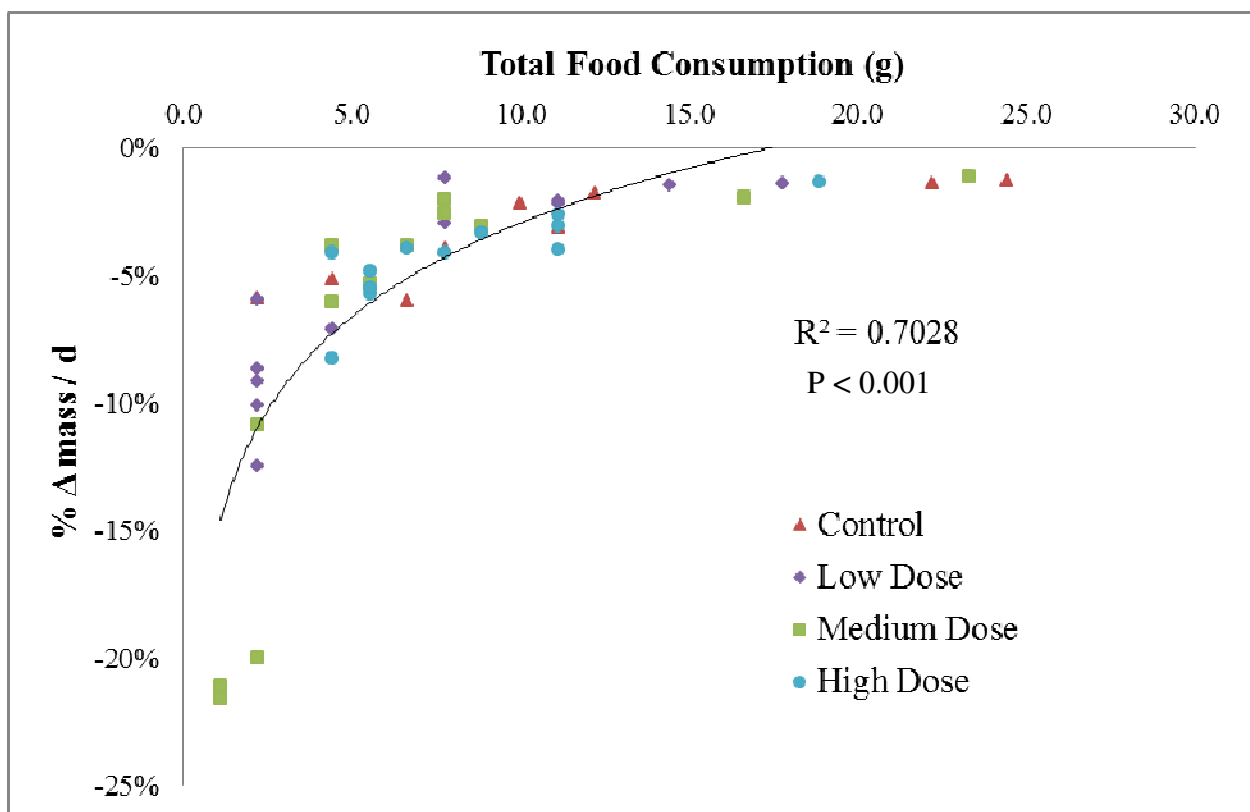


Figure 5. Atlantic Sturgeon Food Consumption and Change in Mass. Relationship between the calculated loss of somatic mass per day and total food consumed for n=48 captive, juvenile Atlantic Sturgeon.

Table 1. *Alosa* spp. Egg and Larvae MC Exposure Experiments. The effects of dissolved MC on the viability of eggs and larvae from American Shad (ASA; *Alosa sapidissima*) and Blueback Herring (AAE; *A. aestivalis*).

Life Stage	Target Concentrations ($\mu\text{g}\cdot\text{L}^{-1}$)	MC	Mean Viable Proportion ^a			
			ASA	AAE 1	AAE 2	AAE 3
Eggs	0.0		0.98	0.91	0.98	1.00
	0.4		0.98	0.93	0.98	0.99
	0.8		1.00	0.91	0.98	0.99
	1.6		0.99	0.91	0.97	1.00
Larvae	0.0		0.96	1.00 ^c	0.99	1.00
	0.4		0.94	0.91	0.99	1.00
	0.8		0.95	0.66 ^d	0.97	0.99
	1.6		0.90	0.69 ^d	0.94	1.00
	3.2 ^b		-	-	-	1.00

^a The response variable is the proportion of eggs or larvae presumably capable of surviving successfully at the conclusion of the exposure (viable proportion).

^b 3.2 $\mu\text{g}/\text{L}$ MC exposure only conducted for trial AAE3.

^c Only a single control bucket recovered for this trial.

^d Low viability likely due to handling stress.

Table 2. Liver MC concentrations of fish following 14 day exposures using two *in situ* enclosures each at the Rice Rivers Center (H) and Curles Neck Creek (L) sites on the James River, Virginia.

Trial	Start Date	Enclosure ID	Species ^a	n ^b	Mean Liver MC Concentration (µg/g DW)
T1	8/9/2013	H1	DCE	15	0.028 ± 0.013
		H2	DCE	15	0.222 ± 0.090
		L1	DCE	15	0.746 ± 0.129
		L2	DCE	15	0.207 ± 0.095
T2	8/23/2013	H1	DPE	4	0.176 ± 0.382
		H2	DPE	2	0.115 ± 0.230
		L1	DPE	6	0.008 ± 0.036
		L2	DPE	14	0.015 ± 0.204
T3	9/6/2013	H1	AAE	4	< 0.001
		H2	AAE	4	< 0.001
		L1	AAE	2	< 0.001
		L2	AAE	0	-

^a Species used were Gizzard Shad (DCE; *Dorosoma cepedianum*), Threadfin Shad (DPE; *D. petenense*), and Blueback Herring (AAE; *Alosa aestivalis*).

^b Represents the number of fish recovered and analyzed at the end of each trial (max = 15) from the initial 30 fish per replicate.

Table 3. Mean MC consumption and liver MC concentrations for captive age-0 Atlantic Sturgeon following 28 days of feeding with MC laced food pellets.

Variable	Treatment			
	Control	Low Dose	Medium Dose	High Dose
Total fish per treatment ^a	9	13	14	12
Mean exposure time(d)	10.1	6.4	6.3	7.6
Concentration of MC in diet (µg/g DW)	0.000	0.014 ^b	0.028 ^b	0.057 ± 0.011
Mean total consumption of MC (µg) ^c	0.000	0.100 ± 0.020	0.197 ± 0.047	0.476 ± 0.068
Mean Liver MC concentration (µg/g DW)	0.000	0.024 ± 0.010	0.041 ± 0.014	0.036 ± 0.027

^a The number of fish per treatment varies due to immediate replacement of all mortalities until the stock tank was depleted.

^b Low and medium concentrations estimated from serial dilution of known high dosage concentration

^c Fish fed a maintenance ration of 1.11 ± 0.05 g of food per day

List of References

- Acuña, S.; Baxa, D.; Teh, S. Sublethal dietary effects of microcystin producing *Microcystis* on Threadfin Shad, *Dorosoma petenense*. *Toxicon*. **2012**, 60 (6), 1191-1202; DOI: 10.1016/j.toxicon.2012.08.004
- Amorim, Á. and Vasconcelos, V. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. *Toxicon*. **1999**, 37 (7), 1041-1052; DOI: 10.1016/S0041-0101(98)00231-1
- Anderson, D.M.; Burkholder, J.M.; Cochlan, W.P.; Glibert, P.M.; Gobler, C.J.; Heil, C.A.; Kudela, R.M.; Parsons, M.L.; Rensel, J.E.J.; Townsend, D.W.; Trainer, V.L.; Vargo, G.A. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae*. **2008**, 8 (1), 39-53; DOI: 10.1016/j.hal.2008.08.017
- Balazik, M.T.; Garman, G.C.; Van Eenennaam, J.P.; Mohler, J.; Woods III, L.C. Empirical evidence of fall spawning by Atlantic Sturgeon in the James River, Virginia. *Trans. Am. Fish. Soc.* **2012**, 141 (6), 1465-1471; DOI: 10.1080/00028487.2012.703157
- Barnhart, M.C. Buckets of mucklets: A compact system for rearing juvenile freshwater mussels. *Aquaculture*. **2006**, 254 (1), 227-233; DOI: 10.1016/j.aquaculture.2005.08.028
- Boesch, D. F.; Brinsfield, R. B.; Magnien, R. E. Chesapeake Bay Eutrophication. *J. Environ. Qual.* **2001**, 30 (2), 303-320; DOI: 10.2134/jeq2001.302303x
- Brooks, M.L.; Fleishman, E.; Brown, L.R.; Lehman, P.W.; Werner, I.; Scholz, N.; Mitchelmore, C.; Lovvorn, J.R.; Johnson, M.L.; Schlenk, D. Life histories, salinity zones, and sublethal contributions of contaminants to pelagic fish declines illustrated with a case study of San Francisco Estuary, California, USA. *Estuar. Coast.* **2012**, 35 (2), 603-621; DOI: 10.1007/s12237-011-9459-6
- Bukaveckas, P.A.; Barry, L.E.; Beckwith, M.J.; David, V.; Lederer, B. Factors determining the location of the chlorophyll maximum and the fate of algal production within the tidal freshwater James River. *Estuaries Coasts*. **2011**, 34 (3), 569-582. DOI: 10.1007/s12237-010-9372-4

- Carmichael, W. Cyanobacteria secondary metabolites—the cyanotoxins. *J. Appl. Bacteriol.* **1992**, 72 (6), 445-459. DOI: 10.1111/j.1365-2672.1992.tb01858.x
- Day, Jr., J.W.; Kemp, W.M.; Yáñez-Arancibia, A.; Crump, B.C. *Estuarine Ecology*, 2nd edition; John Wiley & Sons: Hoboken, New Jersey, **2012**.
- de Figueiredo, D.R.; Azeiteiro, U.M.; Esteves, S.M.; Gonçalves, F.J.M.; Pereira, M.J. Microcystin-producing blooms—a serious global public health issue. *Ecotoxicol. Environ. Saf.* **2004**, 59 (2), 151-163; DOI: 10.1016/j.ecoenv.2004.04.006
- Deblois, C.P.; Giani, A.; Bird, D.F. Experimental model of microcystin accumulation in the liver of *Oreochromis niloticus* exposed subchronically to a toxic bloom of *Microcystis* sp. *Aquat. Toxicol.* **2011**, 103 (1), 63-70; DOI: dx.doi.org/10.1016/j.aquatox.2011.02.006
- Dyble, J.; Gossiaux, D.; Landrum, P.; Kashian, D.R.; Pothoven, S. A kinetic study of accumulation and elimination of microcystin-LR in yellow perch (*Perca flavescens*) tissue and implications for human fish consumption. *Mar. drugs.* **2011**, 9 (12), 2553-2571; DOI: dx.doi.org/10.3390/md9122553
- Eagles-Smith, C.A.; Suchanek, T.H.; Colwell, A.E.; Anderson, N.L.; Moyle, P.B. Changes in fish diets and food web mercury bioaccumulation induced by an invasive planktivorous fish. *Ecol. Appl.* **2008**, 18 (sp8), A213-A226; DOI: 10.1890/06-1415.1
- Ernst, B.; Hoeger, S.J.; O'Brien, E.; Dietrich, D.R. Oral toxicity of the microcystin-containing cyanobacterium *Planktothrix rubescens* in European whitefish (*Coregonus lavaretus*). *Aquat. Toxicol.* **2006**, 79 (1), 31-40; DOI: 10.1016/j.aquatox.2006.04.013
- Gibble, C.M. and Kudela, R.M. Detection of persistent microcystin toxins at the land–sea interface in Monterey Bay, California. *Harmful Algae.* **2014**, 39, 146-153; DOI: 10.1016/j.hal.2014.07.004
- Heisler, J.; Glibert, P.M.; Burkholder, J.M.; Anderson, D.M.; Cochlan, W.; Dennison, W.C.; Dortch, Q.; Gobler, C.J.; Heil, C.A.; Humphries, E.; Lewitus, A.; Magnien, R.; Marshall, H.G.; Sellner, K.; Stockwell, D.A.; Stoecker, D.K.; Suddleson, M. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae.* **2008**, 8 (1), 3-13; DOI: 10.1016/j.hal.2008.08.006
- Hirschfeld, W.J.; Richards, C.M.; Nace, G.W. Growth of larval and juvenile *Rana pipiens* on four laboratory diets. *Amer. Zool.* **1970**, 10 (3), 315.

- Imai, I. and Yamaguchi, M. Life cycle, physiology, ecology and red tide occurrences of the fish-killing raphidophyte *Chattonella*. *Harmful Algae*. **2012**, 14, 46-70; DOI: 10.1016/j.hal.2011.10.014
- Longnecker, M. and Ott, R. *An introduction to statistical methods and data analysis*. Pacific Grove: Duxbury, California, **2001**.
- Malbrouck, C. and Kestemont, P. Effects of microcystins on fish. *Environ. Toxicol. Chem.* **2006**, 25 (1), 72-86; DOI: 10.1897/05-029R.1
- McLean, M.; Dadswell, M.; Stokesbury, M. Feeding ecology of Atlantic Sturgeon, *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 on the infauna of intertidal mudflats of Minas Basin, Bay of Fundy. *J. Appl. Ichthyol.* **2013**, 29 (3), 503-509; DOI: 10.1111/jai.12175
- Miller, M.A.; Kudela, R.M.; Mekebri, A.; Crane, D.; Oates, S.C.; Tinker, M.T.; Staedler, M.; Miller, W.A.; Toy-Choutka, S.; Dominik, C. Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. *PLoS One*. **2010**, 5 (9), e12576; DOI: 10.1371/journal.pone.0012576
- Mohammed, A. Why are Early Life Stages of Aquatic Organisms more Sensitive to Toxicants than Adults? In *New Insights into Toxicity and Drug Testing* [Online]; Gowder, S., Ed.; InTech: Rijeka, Croatia, **2013**; Chapter 3, pp. 49-62. <http://www.intechopen.com/books/new-insights-into-toxicity-and-drug-testing/why-are-early-life-stages-of-aquatic-organisms-more-sensitive-to-toxicants-than-adults-> (accessed February 25, 2015)
- O'Neil, J.M.; Davis, T.W.; Burford, M.A.; Gobler, C.J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*. **2012**, 14, 313-334; DOI: 10.1016/j.hal.2011.10.027
- Oberemm, A.; Fastner, J.; Steinberg, C.E. Effects of microcystin-LR and cyanobacterial crude extracts on embryo-larval development of zebrafish (*Danio rerio*). *Water Res.* **1997**, 31 (11), 2918-2921; DOI: dx.doi.org/10.1016/s0043-1354(97)00120-6
- Paerl, H.W.; Fulton, R.S.; Moisander, P.H.; Dyble, J. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *ScientificWorldJournal*. **2001**, 1, 76-113; DOI: 10.1100/tsw.2001.16
- Pavagadhi, S. and Balasubramanian, R. Toxicological evaluation of microcystins in aquatic fish species: Current knowledge and future directions. *Aquat. Toxicol.* **2013**, 142, 1-16; DOI: 10.1016/j.aquatox.2013.07.010

- R Core Team R: A Language and Environment for Statistical Computing. Vienna, AT. 2014;
<http://www.R-project.org/>
- Schindler, D.E.; Geib, S.I.; Williams, M.R. Patterns of fish growth along a residential development gradient in north temperate lakes. *Ecosystems*. **2000**, 3 (3), 229-237; DOI: 10.1007/s100210000022
- Scott, W.B. and Crossman, E.J. Freshwater fishes of Canada. *B Fish Res Board Can.* **1973**, 184; DOI: 10.1002/iroh.19760610109
- Song, W.; Li, Y.; Fang, S.; Wang, Z.; Xiao, J.; Li, R.; Fu, M.; Zhu, M.; Zhang, X. Temporal and spatial distributions of green algae micro-propagules in the coastal waters of the Subei Shoal, China. *Estuar. Coast. Shelf Sci.* **2014**; DOI:10.1016/j.ecss.2014.08.006
- Stone, H.H. and Daborn, G.R. Diet of alewives, *Alosa pseudoharengus* and Blueback Herring, *A. aestivalis* (Pisces: Clupeidae) in Minas Basin, Nova Scotia, a turbid, macrotidal estuary. *Environ. Biol. Fishes.* **1987**, 19 (1), 55-67; DOI: 10.1007/BF00002737
- Vasconcelos, V.; Oliveira, S.; Teles, F.O. Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkia*. *Toxicon*. **2001**, 39 (10), 1461-1470; DOI: 10.1016/S0041-0101(01)00105-2
- Wilson, E.K. Danger from microcystins in Toledo water unclear. *Chem. Eng. News*. **2014**. 92(32), 9; DOI: 10.1021/cen-09232-notw8
- Wood, J.D.; Franklin, R.B.; Garman, G.; McIninch, S.; Porter, A.J.; Bukaveckas, P.A. Exposure to the cyanotoxin microcystin arising from interspecific differences in feeding habits among fish and shellfish in the James River Estuary, Virginia. *Environ. Sci. Technol.* **2014**, 48 (9), 5194; DOI: 10.1021/es403491k
- Zimba, P.V.; Khoo, L.; Gaunt, P.S.; Brittain, S.; Carmichael, W.W. Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. *J. Fish Dis.* **2001**, 24 (1), 41-47; DOI: 10.1046/j.1365-2761.2001.00273.x
- Zimba, P.V.; Camus, A.; Allen, E.H.; Burkholder, J.M. Co-occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. *Aquaculture*. **2006**, 261 (3), 1048-1055; DOI: 10.1016/j.aquaculture.2006.08.037

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